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The Fate of Plague Bacteriophage in the Organism of Healthy and Plague Infected Great Gerbils and the Possible Routes of Its Transmission in Experimental Conditions.

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From the Central Asian Scientific-Research Antiplague Institute, USSR Ministry of Public Health.

(Submitted 8 May 1961)

In order to obtain a correct concept concerning the interaction of bacteriophage with bacteria in a live organism, it is necessary, first of all, to clarify what is the fate and behavior of phage which is administered to animals by various routes and what are the results of using bacteriophage in various animals with various infections. Several aspects of this problem have been elucidated in the works of d'Herelle (1926), Karpov (1950), Khaykina with coauthors (1955), Petrov (1955), Sutin (1958) and others.

In the present work we attempted to clarify the question concerning the fate of plague bacteriophage in the organism of great gerbils, which are primary carriers of the plague microbe in the Central Asian desert focus of plague. We undertook the following concrete questions: 1) what is the length of stay and localization of plague bacteriophage in the organism of healthy and plague infected great gerbils, 2) what is the fate of the plague microbe in the organism of wild animals injected with bacteriophage, and 3) what are the possible paths of spreading of plague bacteriophage in experimental conditions.

For solving the established problems, experiments were conducted on 290 adult great gerbils which were captured in the sands of Sary-Ishikotrau. From the moment of capture right up to the conducting of the experiments, the wild animals were maintained under laboratory conditions for 14-17 days.

For solving the first problem in the experiment, 210 gerbils were taken, and based on dosages and methods of infection were divided into 7 groups. Of these the last one was the control group.

Plague bacteriophage (titer 10^{-9}) which was prepared by the Central Asian Antiplague Institute was used in the experiments, along with a type culture of plague microbe No 432, which in September 1960 was isolated from fleas.

Unconditionally, the lethal dose of it for white mice consisted of 100 microbial cells. In the cases of infection by bacteriophage and plague causative agents, the first was injected into the right inguinal area and the suspension of plague microbe into the left. After a specified period of time the animals were destroyed and their organs (liver, spleen, lungs, kidneys, adrenal glands, inguinal lymph glands, tissue from the site of administration, blood, urine, bone marrow, and brain) were tested for the presence of phage. In this a direct method of investigation was employed, that is a seeding was conducted with organ and tissue impressions on agar plates which were preliminarily inoculated with a fresh culture of the EV vaccine strain of plague microbe. Along with the infection of animals with a culture of plague microbe and bacteriophage, an additional seeding was carried out on separate agar plates which weren't inoculated with the vaccine strain. Dishes with the inoculations were left in a thermostat at 30-32°. The results of phage lysis were checked after 48 hours. In the absence of the phenomenon of phage lysis, some segment of the dishes were maintained for 4-6 weeks.

The results of the experiment showed that the length of preservation of bacteriophage in great gerbils depended on the dose, the method of administering the bacteriophage, and on their infection with a culture of plague microbe.

With a subcutaneous administration, the bacteriophage was preserved only up to seven days, but with an increase of the dose from 1 to 5 ml the frequency of its isolation doubled. In gerbils infected with bacteriophage and plague bacilli, phage was isolated from the liver, spleen, and lymph nodes up to 19 days. Bacteriophage wasn't isolated from animals which were infected with phage perorally.

It must be noted that phage was isolated in two gerbils which were infected only with a culture of plague microbe. This problem requires further study.

The second division of work was the study of the fate of the plague microbe in the organism of gerbils infected with phage.

There is already some material in literature on the study of the interaction of the microbe with phage in the organism of animals.

We took 90 animals for solving the problem of the possible influence of bacteriophage on the plague microbe in the organism of great gerbils. After an analysis of material obtained it was established that out of these animals 42 cultures of plague microbe were isolated, while colonies infected with phage were isolated from 18. We didn't observe the phenomenon of bacteriophagy in cultures isolated from all the inoculations of the internal organs of the same rodent, but most of all we isolated phage infected cultures from the spleen, liver and lymph nodes. The degree of phage lysis of colonies even of the same inoculation was unequal.

In five gerbils phage infection of colonies appeared when the dishes had been standing in the thermostat for a longer period (20 - 32 days).

The phage infection of colonies of plague microbe in gerbil No 78 (see drawing) is interesting. This animal was infected subcutaneously with a suspension containing 100 million microbial cells of plague microbe and 24 hours after infection received 1 ml of bacteriophage. The gerbil died on the third day. Upon autopsy, hyperemia of subcutaneous vessels and enlargement of the liver and spleen were noted in it. In seedings on agar plates preliminarily inoculated with vaccine strain, the phage was established in all the internal organs and also in the blood and urine. Phage wasn't detected in the bone marrow and brain. In seedings from the specified organs and tissues, side by side with the changed colonies, typical colonies were found which in the subsequent storage of the dishes were subjected to phage lysis. Cultures obtained from seedings of the liver and spleen were the most infected with phage ("ghost colonies"). In reinoculations of "ghost colonies" on agar plates with antiphage serum, better results were obtained than in reinoculations on ordinary Hottinger agar.

In connection with the fact that the great gerbils in nature live with large families and are parasitized by a large number of fleas, we were interested in the problem of the possibility of bacteriophage transmission during their common maintenance, having in mind the possible transmission through feed, excrement, and also with the help of fleas.

Experiments were carried out on 80 rodents. Before the experiment 40 adult animals were dusted in order to exclude the possibility of transmitting phage by the transmissive route. The gerbils were divided into two groups (20 animals in each) and for the extent of 10 days were maintained in two wooden boxes which were lined internally with iron. Then five animals of the first group were infected subcutaneously with 1 ml of bacteriophage and five gerbils of the second group - with bacteriophage and plague microbe (100 million microbial cells). The animals were killed at various intervals up to 12 days. Phage was isolated only in preliminarily infected animals - in 2 animals of the first group and in 1 animal of the second group (up to 7 days). Consequently the transmission of phage during the common residence of gerbils was not established by us.

The following work was carried out for solving the problem of the possibility of transmitting the phage through fleas. We didn't dust the second segment of gerbils (40) and also divided them into two groups. Then five gerbils of the first group were infected subcutaneously with 1 ml of bacteriophage, and five gerbils of the second group were each administered subcutaneously 1 ml of phage and 100 million plague microbes. Two hours after infection 200 Xenopsylla gerbilliminax fleas were liberated on the animals in each of the two groups. The animals were killed at various intervals (up to 20 days). Before autopsy the animals were combed clean. In all, 23 fleas from the first group were investigated for the presence of bacteriophage, and 14 from the second group.

From the first group of animals phage showed up in one which was infected earlier and killed on the fifth day, and also in two fleas. In the second group it appeared in two gerbils which we had subjected to infection earlier and in six fleas taken from healthy and infected animals. Consequently we didn't establish the transmission of bacteriophage with the help of the fleas either.

The data obtained indicates that by the direct method of detecting bacteriophage it is impossible to establish its dissemination under experimental conditions. If it was capable of spreading, then it is in very small (perhaps unsuitable for detection by any method of investigation) quantities which could rapidly be excreted from the organism or rapidly become inactivated. Therefore it should be assumed that apparently the plague bacteriophage has an endogenous origin and it is necessary to study those conditions which cause its appearance in the organism of great gerbils and their fleas.

Since, as Tiflov showed (1948), phage can circulate in the blood of gerbils for a long time, in satiated and infected fleas under the action of the phage their sterilization from plague infection can result.

Conclusions

1. During the subcutaneous application of plague bacteriophage it is preserved in the organism of great gerbils up to 7 days, and in animals infected with plague, - up to 19 days. The frequency of isolating phage depended on the dose of phage administered and on the infection of the gerbils with a culture of plague microbe.

2. For detection of phage under field conditions it is necessary to investigate the spleen and lymph node where it is found more often and for a longer time.

3. Bacteriophage shows a lytic action in the organism of great gerbils, but not to such a degree as on artificial nutrient media.

4. Upon the investigation of inoculations from field material and the detection of "ghost colonies", the latter must be reinoculated on agar plates with antiphage serum.

5. With the aim of detecting bacteriophage by the direct method of investigation, it is necessary that the inoculations be maintained in a thermostat for a long time (in a number of cases up to a month).

6. By the direct method of investigation the transmission of plague bacteriophage was not established among great gerbils during their common residence and by way of fleas.

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Picture caption (page 33): 48 hour phage infected colonies of plague microbe, isolated from great gerbil No 78.
1-2 - phage infected colonies from the liver.
3-4 - phage infected colonies ("ghost colonies") from the spleen, x76.

[Following is the English summary which appears with the Russian original.]

On the Fate of Plague Bacteriophage in the Organism of Healthy and Plague Infected Rhombomys and the Possible Routes of Its Transmission in Experimental Conditions.

I. L. Martinevsky, M. A. Shashaev, N. F. Tarakanov and A. T. Shapovalov

Experiments were staged on 290 Rhombomys. As established, plague bacteriophage infected subcutaneously was retained in the organism of healthy animals up to 7, and in the plague infected animals up to 19 days; it was present in the spleen and lymph node for the longest time. Bacteriophage produced a lytic action on Past. pestis in the organism of investigation, it is necessary to keep the cultures in an incubator for a long period of time (up to a month). Spread of plague bacteriophage among the Rhombomys or transmission through fleas was not noted.